OBSERVATIONS ON THE STRUCTURE OF PROTOPLASM BY AID OF MICRODISSECTION.

WILLIAM SEIFRIZ.

TECHNIQUE.

The introduction of the Barber pipette holder brought into use an exceedingly ingenous technique which promises to open up a great field of research, in which not only many of the results got from fixed material can be confirmed or refuted, but in which observations on the structure and behavior of living protoplasm can be made with an accuracy and certainty not otherwise possible. This instrument, originally designed to hold miniature pipettes for isolation and injection work, is equally well adapted for the manipulation of glass dissection needles. Each needle is held in a three-movement clamp and extends into a small moist chamber placed on the stage of the microscope. The vertical needle-tips project up into a hanging drop in which the material to be dissected is suspended.

A description of this instrument and of the technique connected with it was first published by Barber (1914). Chambers (1915) has given a brief account of its use, and later (1917*c*) published a full description of the instrument and of the ways of making micro-needles.[1]

LITERATURE.

The literature published on microdissection studies is limited practically to the articles of G. L. Kite and Robert Chambers. Both of these men employed a Barber instrument essentially identical with that used by the writer. A list of their publications is appended to this paper.

TERMINOLOGY.

A serious difficulty encountered by the investigator in this field is the limited vocabulary which is at his disposal for the

[1] It is of great importance in making sharp needles to have a minute flame. A very satisfactory micro-burner can be made—as was suggested to the writer by Dr. E. E. Free—from the smallest size (no. 27) hypodermic injection needle by snipping off the bevelled end with sharp shears.

definition and description of the phenomena observed. We are little better off in choice of words than were the early microscopists whose knowledge of protoplasm was fully indicated by such adjectives as viscous, elastic, hyaline, etc.

In the possible range in consistency between the two extreme conditions of a colloid, *i. e.*, extreme liquidity and the solid state, the writer will distinguish ten degrees of viscosity, namely—watery, very liquid, liquid, slightly viscous, rather viscous, decidedly viscous, very viscous, extremely viscous, gel, and rigid gel—and has employed these expressions, for want of better ones, to describe the comparative viscosity of protoplasm. Their use will eliminate, in part, the vagueness from such general terms as liquid and viscous.

Protoplasm, which includes "all the living components of the cell-body" (Strasburger, 1891, p. 13), possesses many lifeless inclusions which materially affect its consistency, and they must be taken into consideration when the viscosity of protoplasm is discussed. As hyaloplasm is usually restricted to the hyaline border of non-granular plasma, the term matrix is used to indicate the translucent and more homogeneous fluid in which the various included granules are suspended.

Protoplasm, as such, frequently possesses a consistency different from that of its matrix. The latter may be watery yet the density of the protoplasm in toto markedly greater. It is, therefore, important to keep clear the distinction between the protoplasm as a whole and the matrix in which the various inclusions are imbedded.

MATERIAL.

The experimentation leading to the following results was carried on in the Harpswell Laboratory at South Harpswell, Maine. The writer wishes to thank the director, Dr. J. S. Kingsley, for the use of a room in the laboratory and for many other privileges enjoyed there.

The chief problem for the botanical microdissectionist is the obtaining of material delicate enough to permit dissection by fine glass needles. Even the wall of the frail alga *Spirogyra* can be entered only by the sharpest and most substantial needle that can be made. All in all, the most satisfactory objects for both zoö-

logical and botanical workers are the ova of marine organisms, although the myxomycetes are in some respects still better, for we have in them the largest masses of pure protoplasm known.

The observations here published were made upon myxomycetes, pollen-tubes, the oögonia, ova, and embryos of *Fucus*, and the ova of *Echinarachnius*.

OBSERVATIONS.

MYXOMYCETES.

Plasmodia of *Ceratiomyxa*, *Badhamia*, *Arcyria*, *Cribraria* and *Fuligo* were studied.

The protoplasmic density of myxomycetes is such that a needle traverses the plasma as through water, although it exhibits a slightly viscid property, for inclusions are pushed ahead of and to the side of a needle before they actually come in contact with it. However, if the point of a needle is broken off, making of it a minute pipette, cytoplasm and small inclusions rush into the opening with great rapidity and from quite a distance.

The vegetative plasmodium of a myxomycete is of very liquid consistency and remains so no matter how thin the film or filament of streaming plasma may be. If the plasmodium is not active a film or isolated globule of its protoplasm gels very rapidly. The consistency becomes so dense that a moving needle leaves a permanent furrow.

The old appellation "naked protoplasm," much used in reference to the slime-moulds, is in important respects a misleading one. The surface layer of a plasmodium is a definite morphological structure. The membrane is very extensile, slowly contractile, and surprisingly tenacious for so delicate a layer. This superficial layer can be isolated and held by one needle while stretched to several times its length by the other.

Within the plasmodium are definitely bounded smaller masses of protoplasm, which are apparently normal and in every respect identical with the surrounding plasma bulk. The origin of these smaller included protoplasmic masses was not observed, but their presence is common and they are readily distinguished from oil or other liquid inclusions. Several of these globules were isolated and dissected. Their limiting membrane is exceedingly sensitive,

breaking before a perceptible indentation can be noted. Further dissection gave convincing evidence that this membrane is a morphologically differentiated layer. On breaking a globule some of the liquid protoplasm escaped. The pellicle of the ruptured mass stood out prominently and could be handled with a needle as one would handle a hair. It was of no appreciable thickness, yet quite rigid, though easily bent with no indication of being soft or glutinous. It had, therefore, during dissection, undergone a change—the normal, and extremely sensitive membrane had become a tough rigid gel. Such behavior well supports our interpretation of the plasmodium pellicle—not a surface-tension membrane nor a secreted wall, but a bounding layer of denser protoplasm. The plasmodial membrane is, then, a gel of appreciable thickness, tenacious, very extensile, contractile, and glutinous.

Next in importance to the possession of a definite morphological membrane is the capacity for forming one. The evidence which dissection work on myxomycetes presents on this phenomenon is, briefly, that the membrane is instantly and repeatedly reformed when ruptured by a needle, provided the protoplasm is normal. This is true whether the dissection be performed on a dry cover or in water; that is, the capacity is unchanged whether air or water is the surrounding medium. If gelation has set in the capacity for membrane formation is lost, although it may persist surprisingly long.

The living substance of slime-moulds is non-miscible in water.

POLLEN TUBES.

Of the many pollen grains experimented with those of the large blue flag (*Iris versicolor*) were the most satisfactory. The grains are large and germinate readily in almost any per cent. of sugar solution. The pollen of the beach pea (*Lathyrus maritimus*) is a fair substitute.

Repeated irritation of a pollen tube puts an end to protoplasmic streaming, although in some instances streaming may continue even after a tube has been punctured, and a large amount of its contents lost. Streaming, however, is not accelerated by irritation, nor is there any indication of a rush of protoplasm to

wounded regions. The active protoplasm of a pollen tube is of very liquid consistency. On ejection it gels, though it does so slowly. Brownian movement sets in shortly after.

Ejected masses of protoplasm from pollen tubes develop membranes immediately on being freed. The capacity for membrane formation persists one to two minutes. Within three minutes the escaped protoplasm has become quite viscous. The membrane formed is surprisingly tough. Fragments of it can be dragged into the escaped plasma mass.[1]

Escaping protoplasm shows no sign of miscibility. Isolated groups of inclusions exhibiting Brownian movement, after ejection, were carefully observed and in every instance the cytoplasmic matrix was distinctly visible.

FUCUS.

The eggs of *Fucus* develop from divisions of the contents of oögonia which arise from single superficial cells of the wall of conceptacles that cover the fruiting branches. If these oögonia are teased out at a very early age they can be entered by a sharp needle. Very soon, however, the outer wall (exochiton) becomes too hard to be penetrated.

Oögonia.

In consistency the protoplasm of young uninucleate oögonia is very liquid. The wall is thick (2–4 microns), tough, and highly resilient. Slightly older oögonia, but still uninucleate, also possess very liquid protoplasm. Stages in development between young uninucleate oögonia and almost mature oögonia can not be observed, as the tough outer wall does not permit of dissection.

Immature Ova.

The contents of nearly mature oögonia can be squeezed out of the heavy exochiton by crushing the conceptacle with tweezers.

[1] It was the intention of the writer to investigate the structure of the vacuole, of which so many are produced in the growing pollen tube. Freed vacuoles hold their shape even where exposed around the border of the plasma mass, apparently free of surrounding cytoplasm. When punctured the vacuoles collapse immediately. This behavior suggests that there is an enclosing membrane, and thus that the vacuole is apparently not really a vacuole but a sac. (De Vries, 1885, p. 467.)

Unripe eggs obtained in this manner are still held together by a jelly mass. The earliest stages so obtained were of oögonia in which division was complete but the eight eggs still of pentagonal outline in profile and closely appressed.

The protoplasm is of liquid consistency but shows marked signs of an increased viscosity over that of young oögonia. It tolerates a great amount of ill-treatment without showing any signs of injury. After half an hour of dissection there was no indication of gelation, the viscosity of the protoplasm remaining the same. Brownian movement, that unfailing criterion of degeneration, was not seen.

Slightly older oögonia in which the eggs, though still closely appressed, have rounded up somewhat, show further increase in protoplasmic consistency to the slightly viscous stage.

The separating membranes of these closely appressed masses of protoplasm are exceedingly delicate and of inappreciable thickness.

The capacity for membrane formation is complete. A remarkable property of the protoplasm of a young *Fucus* ovum is the rapidity with which it is enclosed by a wall after a needle has severed the egg. The very elastic glutinous membrane, ordinarily barely visible, is sufficiently pliable to be made of appreciable thickness when pressure is applied laterally with a tendency to compress.

The above described behavior of the ripening *Fucus* egg leaves no doubt that the plasma is a non-miscible fluid.

The stage of development of the eggs just described must be borne in mind. These ova, of slightly viscous consistency and enclosed by a delicate membrane, are nearing the completion of their development. They are still closely appressed within the oögonium, with several hours intervening before they would have been discharged as ripe eggs with gelatinous contents and a thick, hyaline wall.

The protoplasm of eggs of more advanced oögonia is of greater density, namely, rather viscous. It flows readily, but slowly.

The last stage in the development of the eggs before their discharge is especially noticeable because of an increase in thickness of wall. The enclosing layer is now one half of a micron

thick. Notwithstanding the possession of a heavier wall these eggs can be dissected with as much ease, ending in the same results as those less mature ova having but a thin pellicle as covering. Droplets can be pinched off, the eggs torn from within outwards, or rapidly severed by a needle, without any indication of escaping protoplasm. The severed parts in every case instantly round up into droplets with walls apparently identical with the parent wall. This capacity to form a wall instantly at a ruptured point through conversion of the cytoplasm into a semi-rigid gel is not influenced by relatively great changes in the concentration of the surrounding medium, for it is as pronounced in very saline water as in normal sea-water.

There is no evidence of miscibility on the part of the protoplasm.

Fully Mature Ova.

The protoplasm of mature, normally discharged eggs is decidedly viscous, noticeably more so than that of well-developed unripe eggs. Thus has the highest degree of viscosity been reached in the development of the ovum, the transition taking place during the last periods of growth.

The wall likewise has undergone a marked change and become a hyaline, rigid gel, 0.8–1.2 microns thick, still very pliable and extensile, slightly contractile and exceedingly adhesive.

The wall of the mature normal *Fucus* ovum is capable of constant repair and this capacity often persists to the very last in a dying egg. Not until gelation of the protoplasm is well advanced does a rupture of the wall fail to be closed by a rapid conversion of the plasma matrix into a rigid gel. The capacity for wall formation is, then, one of the last essential properties of the living substance to be lost.

In all stages of its development before its normal discharge the egg shows no injury in consequence of dissection. The mature egg, on the contrary, is very sensitive to dissection, although its behavior is extremely variable. Every precaution was taken to prevent a misinterpretation of results due to observation of degenerated protoplasm. Such precaution was found to be more necessary with the ova of plants than with those of animals. To determine that the ripe eggs used for dissection

were normal, part of each lot used was kept and tested for fertility.

The response to dissection of ripe unfertilized ova may vary from immediate disintegration to the toleration of two severances. Many eggs are very easily pinched in half by two needles rapidly approaching from opposite sides. The halves frequently round up into perfect spheres which can be again severed to form smaller protoplasmic droplets. While some eggs will show but slight if any increase in viscosity after many minutes of slow movement of the needles, others will suffer little or no dissection before gelating or completely disintegrating. Mere puncturing of the egg will often cause gelation. Thus does the ripe egg exhibit in one instance great sensitiveness and in another apparent indifference to stimulus.

This variability is carried even to parts of the same egg. One half of a severed ovum may gelate immediately without forming a wall over the torn surface, while the other half tolerates still another severance before gelating and thus losing the capacity for wall-formation. The presence of the nucleus in one half of the egg may be responsible for this difference in behavior (Townsend, 1897), although frequently both halves develop enclosing walls.

The protoplasm of the living ovum is at all times non-miscible in sea-water. This is to be expected if the capacity for wall-formation persists as long as the protoplasm is alive. In no instance of eggs examined in the various stages of ripening, while mature, and in the brief period following fertilization when the wall was still penetrable, was the plasma found to pour out and mix with the surrounding water.

The Unicellular Embryo.

Fertilization in *Fucus* is readily accomplished if active sperm are placed on a slide with mature ova.

Half an hour after application of the sperm the protoplasm of the fertilized ovum is found to be still quite viscous. A little later it becomes more liquid, for it readily oozes out of a puncture. Further development of the unicellular embryo shows a continued decrease in protoplasmic density.

The wall immediately after fertilization shows no increase in thickness and is still very pliable, extensile, and contractile. A little later it stiffens up until no longer elastic, though slightly resilient and pliable in so far that permanent indentations can be made. It can not now be severed by two approaching needles. In those few instances where the tough wall was punctured, and the protoplasm forced out, the ejected mass immediately developed a membrane. This plasma membrane is exceedingly thin, and quite elastic. It is interesting to note that the freed protoplasm here forms a delicate pellicle—not the tough wall of the one-celled embryo from which it came, nor the substantial though glutinous egg-wall which it would have developed in repairing a tear before fertilization.

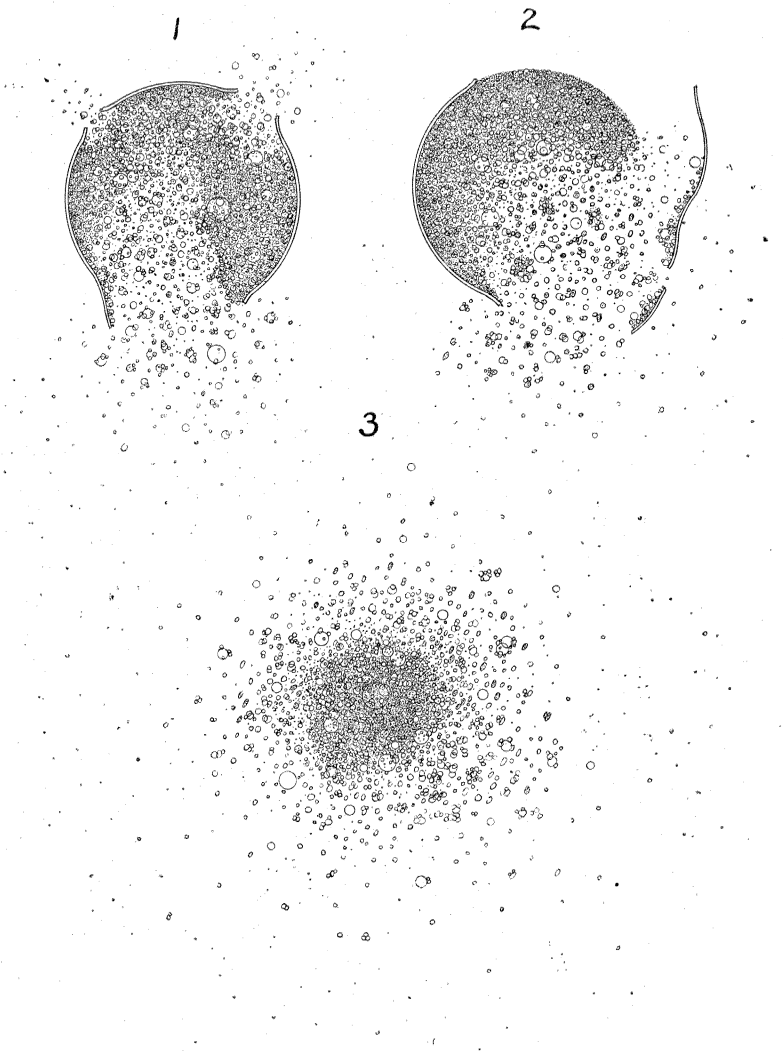
At all times, from the moment of fertilization until dissection is no longer possible, the embryonic protoplasm is non-miscible. The ejected plasma shows no tendency to mix with the surrounding sea-water until after gelating, *i. e.*, after death.

The Multicellular Embryo.

As the two-celled embryo stage is neared the former egg wall has lost all of its former elasticity and glutinosity. It behaves under pressure like a ball of thin celluloid, resisting light pressure and returning to its former shape, unless forced beyond a certain point, when depressions made are retained. The two-celled embryo is punctured with great difficulty. The wall of the four-celled embryo is a very substantial affair, two microns in thickness, highly resilient and impenetrable to the sharpest needle.

The Degenerate Egg.

The behavior of the over-ripe ovum is extremely interesting and instructive in its bearing on the mechanism of the egg contents. As already stated, an ovum ultimately gelates and usually disintegrates. A gelated egg may be torn apart with great ease. If hardening has progressed far enough the egg may be cut in pieces as one would cut butter. If, on the other hand, the contents are not too solid the matrix may be readily drawn out into a long invisible thread. It is this gelated cytoplasmic matrix which is water-miscible.



DESCRIPTION OF DRAWINGS.

FIG. 1. A disintegrating *Fucus* ovum in which smaller craters have burst forth subsequently to several seconds of activity on the part of the larger crater.

FIG. 2. A degenerate *Fucus* ovum in which discarded fragments of the wall are seen, and in which dissemination of granules is very active in one region while non-existent in another.

FIG. 3. The wild and rapid scattering of the inclusions of an exploded *Fucus* egg.

The disintegration of a degenerate egg is accomplished by the matrix going into solution with the sea-water. This may take place with such rapidity as to suggest a miniature explosion of the egg; or may be delayed and then it usually proceeds slowly, either in spasmodic periods of dissolution, or continuously until the entire granular contents has been disseminated. Often this is never completed. Where the disintegration is instantaneous (Fig. 3) it takes place immediately on the over-ripe egg coming in contact with a needle, or by the egg itself before actual dissection can be got under way. The miscibility of the matrix may be general (Fig. 3), or localized (Fig. 1). Where dissolution takes place without dissection some internal pressure, evidently osmotic, brings about a rupture. But it is difficult to see why such a force does not greatly expand so pliable a wall before breaking it. This is not the case. Furthermore, where disintegration has been instantaneous wall fragments are not to be found. When the process is gradual bits of it are only occasionally seen (Fig. 2). There is some reason for believing that the pliable, glutinous wall itself undergoes a change during degeneration of the protoplasm. If it does not go into solution it probably becomes a soft, inelastic gel.

A distributed, internal, osmotic pressure would be relieved once the egg-wall had broken at any one point, and one would not expect further eruptions. Quite the contrary is true. There may be a rather general dissemination of granules from one region for several seconds, time enough to relieve any pressure within, and subsequently, other craters break forth as shown in Fig. 1. These are localized centers of activity. That scattered regions of the egg contents are in different physiological states is further evident from the fact that certain localities may from the very outset remain inactive, never taking part in the general dissolution of the protoplasm, such as the exposed upper surface in Fig. 2.

The force responsible for a wild scattering of the inclusions in an ovum is a surface-tension one, due to an extraordinarily rapid miscibility of the degenerate matrix in the surrounding medium of sea-water.

ECHINARACHNIUS.

Microdissection work on the egg of the sand-dollar was undertaken for the purpose of comparison with the behavior of the plant ovum.

The difference in structure and behavior of *Fucus* and *Echinarachnius* ova before fertilization is not great. With fertilization two striking dissimilarities originate. There is in *Echinarachnius* an increase in viscosity of the protoplasm after fertilization. The path of a needle closes very slowly. Furthermore, there is very little change in the character of the egg wall. The wall of the young embryo is of no greater thickness than that of the ovum and but slightly more resistant.

The *Echinarachnius* egg occasionally tolerates a very great amount of dissection before completely deteriorating. At times, the capacity for wall formation persists in a region of still liquid plasma even after other regions of the egg have become an exceedingly viscous mass. This illustrates further the great diversity in the behavior of the protoplasm of eggs existing under apparently identical environments, and the marked difference in physiological condition of different regions of the same cell.

During the dissection of more than 20 eggs of the sand-dollar but one instance of miscibility was observed, and this was the rapid dissemination of granules due to the going into solution of the matrix of a gelated, that is, a degenerating ovum.

DISCUSSION.

In spite of the variety of objects studied and the differences in behavior of very similar material there are certain definite properties which characterize all the protoplasm here observed.

Streaming protoplasm is of a very liquid consistency. The same is true of young, actively growing plasma such as that found in the developing oögonium and the embryo of *Fucus*. The increase in consistency of the *Echinarachnius* ovum following the entrance of the sperm is not in harmony with this fact, nor is it what one would expect; for the liquid condition of protoplasm enables the elaborate chemical reactions of an active cell to take place. In the ripe egg, awaiting fertilization, metabolism is reduced to a minimum, so that a liquid state is not needed as it

is for the complex chemical activities and interchange of substances which go on in the developing egg and growing embryo. In accordance with this is the fact that normal passive protoplasm in mature eggs of *Fucus* and *Echinarachnius* ova is quite viscous. (In ripe and resting seeds the protoplasm becomes almost solid.)

There is no reason for believing that any living protoplasm is naked. The surface membrane of protoplasm is at all times a definite morphological structure. The capacity to form such a pellicle is one of the characteristic properties of the living substance and is retained to a very late stage in dying protoplasm.

The membrane formed in repairing a tear is of the same character as the original. This is not true in those instances where the wall is of cellulose, as in pollen-tubes and plant embryos; for the enclosing surface layer of protoplasm is but a transformed portion of the living substance, the result of an immediate conversion of liquid plasma into a rigid gel of greater molar concentration. (Pfeffer, 1891, p. 194). It is worthy of note that protoplasm possesses not only the capacity to form a membrane but that kind of a membrane characteristic of a particular organism or of a particular stage of development. This suggests that the process is to some degree controlled from within.

That contact with a *certain* medium is not necessary for membrane formation is evidenced from the fact that streaming myxomycetes form membranes whether dissected on a dry coverslip or in a hanging drop, and that the pellicle of the *Fucus* ovum is repaired whether it is torn open in the jelly-mass of the oögonium or in sea-water. Thus does it appear that contact with some medium is not a prerequisite to membrane formation, the lack of a membrane being the stimulus. Membranes are formed on any free protoplasmic surface. The general belief is that this is a purely physical process, and owes its origin to surface forces. It is somewhat disturbing to this strictly mechanistic conception that the capacity for membrane formation ends with death. (Pfeffer (1877) has shown experimentally that a membrane may be formed after death. Chemistry could give us many such instances. In neither case is the membrane a natural one.) Surface forces certainly come into play, but the capacity for membrane formation, and beyond doubt the factor determining

the kind of membrane formed, is dependent upon the physiological state of the protoplasm, which, in a sense, means that the ultimate control lies within the cell; though, of course, not beyond the purely physical and chemical properties of the living substance.

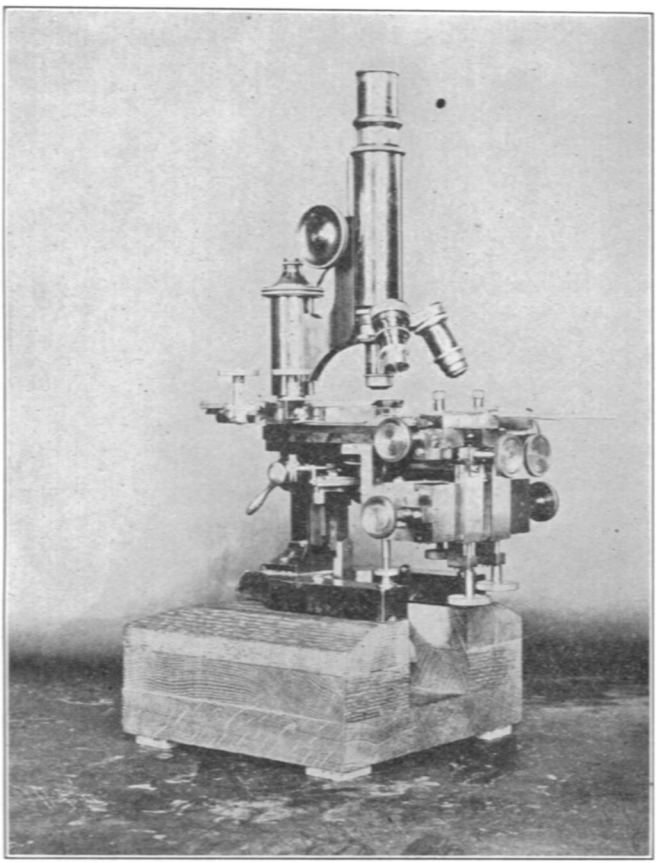
The prevailing idea of a cell wall is that it is a dead structure and, therefore, incapable of change or response to environment. This is probably true of the cellulose walls of older cells, but not of the distensible, glutinous membranes and egg-walls here discussed nor of the cell-walls of meristematic tissues in general. The suggestion that the wall of a degenerating ovum may and does undergo, with the egg-protoplasm, a change which permits either its going into solution or its ready rupture by internal osmotic pressure, is not without experimental support. Protoplasmic membranes are an intimate part of the living substance and susceptible to the same changes in environment. (Bayliss, 1916, p. 115.)

Normal protoplasm, in all cases studied by the writer, does not mix with water. This declaration is contrary to that of Chambers (1917*a*, p. 2). If the decision is to rest on evidence gained solely from microdissection then miscibility of the protoplasm is a consequence of degeneration. Chambers's statement, however, rests not only on microdissection but on the introduction of water into the cell by the mercury injection method as well. Miscibility precludes the presence of a membrane. Normal protoplasm is always capable of membrane formation. Therefore, normal protoplasm can at no time be miscible. The dissection of a great many eggs supports this conclusion.

To the highest powers of the microscope, with ordinary illumination, protoplasm is a homogeneous, structureless solution. Its colloidal nature becomes evident on using the dark ground illuminator. Protoplasm is probably an emulsoid hydrosol, *i. e.*, a colloid in which both phases are liquid, one of them—the dispersion medium—being water.

Any definite statement on the ultimate structure of protoplasm must be expressed in physicochemical terms, and based upon observations made with the ultra-microscope. The behavior of protoplasm under dissection, however, throws considerable light on the gross structure of the cell contents. In dis-

tinguishing between gross and ultimate structure the writer has in mind, under the former, a haphazard arrangement of the minute subdivisions or centers of activity, of the living substance which are at any one moment in differing physiological states;



DESCRIPTION OF PHOTOGRAPH.

A two-needle Barber pipette holder attached to a Leitz microscope.

while an ultimate structure connotes a definite arrangement of molecules or of the infinitesimal particles seen through the ultramicroscope—the microns or inferred amicrons of which all colloidal systems are composed.

The most remarkable feature of life within a cell is the coexis-

tence of a great number of reactions, constantly going on without one interfering with the other. This demands the division of the contents into innumerable chambers or centers of activity. Thus does the protoplasm possess, in a sense, structure. The presence of these miniature laboratories is well illustrated by the behavior of disintegrating ova in which the dissemination of the inclusions is at times in certain regions extremely rapid, and in others non-existent (Figs. 1 and 2). The difference in the physiological state of the protoplasm at any one moment is responsible for the variety of behavior of eggs from the same oögonium and of parts of the same ovum. This difference is to be expected if protoplasmic activity takes place in pulsations, which are neither synchronous among the eight eggs of one oögonium nor rythmic in any single ovum. The cell is, then, a laboratory in which many different chemical reactions are constantly going on, kept free from one another by boundaries of some kind. (Hofmeister, 1901.)

Structure in protoplasm is secondary to activity. Upon the chemical nature of the substances does the life of the cell depend rather than on their arrangement. What physical structures may exist are of a transitory nature. This interpretation does not preclude the all-important organization upon which the continuance of these activities depends.

SUMMARY.

1. Protoplasm is an emulsion colloid normally in the sol state.

2. The density of protoplasm varies from the very liquid state of young *Fucus* oögonia and embryos and of streaming protoplasm in myxomycetes and pollen tubes, to the quite viscous condition found in mature and resting eggs of marine organisms.

3. There is a rapid increase in viscosity of the *Fucus* egg during the last stages of its ripening, which is, on fertilization, followed by a return to the liquid consistency characteristic of active, growing proptolasm.

4. The plasma membrane is a definite morphological structure, constantly and repeatedly capable of repair through the conversion of the fluid protoplasm into a hyaline layer of greater molar concentration. This film of gel is exceedingly elastic, pliable and glutinous.

5. The surface layer, like the interior cytoplasm, seems to be capable of alteration with changes in environment.

6. The capacity for membrane-formation is one of the last essential properties of the living substance to be lost. It is lost only at death.

7. The kind of membrane formed is apparently identical with the parent membrane (except in the case of escaped protoplasm from cells which possess a cellulose wall).

8. The formation of a membrane is probably a purely physical process, but is dependent upon the physiological condition of the protoplasm. It is not dependent upon the surrounding medium.

9. The amount of physical disturbance that protoplasm can be subjected to before showing signs of injury varies from that of the immature *Fucus* ovum, where it is exceedingly great, to that of the ripe egg where it is very slight, often no more than a touch sufficing to cause disorganization.

10. Gelation of the plasma always takes place in time and is hurried by dissection. It is accompanied by degeneration.

11. Normal protoplasm is at all times non-miscible in water. Miscibility of the plasma is an unfailing criterion of degeneration.

12. Dissolution of the *Fucus* ovum is the result of the disorganized cytoplasmic matrix going into solution with the surrounding water. This mixing may take place with the rapidity of an explosion, or slowly, and then either continuously or spasmodically.

13. The disintegration of the egg plasm is frequently localized, in that certain regions of the contents continue dissemination of the granules from the beginning, while others join in later, and still others never take part. This indicates a gross structure of the egg plasm, *i. e.*, the protoplasm is composed of many centers of activity in which different chemical reactions take place separated by protective partitions.

14. That any definite and permanent arrangement of the colloidal particles exists seems unlikely. Whatever structure, gross or ultimate, protoplasm may possess is secondary to chemical activity upon which the life of the organism depends.

The writer wishes to thank Professor Duncan S. Johnson for suggesting this study and for assistance during its progress.

To Dr. Robert Chambers the writer is indebted for helpful suggestions, and to Dr. E. V. Cowdry for the use of his Barber instrument.

BIBLIOGRAPHY.

Barber, M. A.

'14 The pipette method in the isolation of single micro-organisms and in the inoculation of substances into living cells. The Philippine Jour. of Sc., Vol. 9, Sec. B, Tropical Medicine, p. 309.

Bayliss, W. M.

'15 Principles of General Physiology. London: Longmans, Green, and Co.

Chambers, R.

'15 Microdissection studies on the physical properties of protoplasm. Lancet-clinic. Mar. 27, 1915, Cincinnati.

'17a Microdissection Studies. I. The visible structure of cell protoplasm and death changes. Amer. Jour. Physiol., Vol. 43, p. 1.

'17b Microdissection Studies. II. The cell aster: a reversible gelation phenomenon. Jour. Exp. Zoöl., Vol. 23, No. 3.

'17c The Microdissection Method. BIOL. BULL., Vol. 32.

De Vries, Hugo

'85 Plasmolytische Studien über die Wand der Vacuolen. Jahrbücher für wissenschaftliche Botanik, vol. 16, pp. 465–598. Berlin, 1885.

Kite, G. L.

'12 The nature of the fertilization membrane of the egg of the Sea Urchin (*Arabacia Punctulata*). Science, N. S., Vol. 36, No. 930, p. 562.

'13a The relative permeability of the surface and interior portions of the cytoplasm of animal and plant cells. BIOL. BULL., Vol. 25, No. 1, p. 1.

'13b Studies on the Physical Properties of Protoplasm. I. The physical properties of the protoplasm of certain animal and plant cells. Amer. Jour. Phys., Vol. 32, No. II.

Kite, G. L., and Chambers, R., Jr.

'12 Vital staining of chromosomes and the function and structure of the nucleus. Science, N. S., Vol. 36, No. 932, p. 639.

Hofmeister, Franz

'01 Die Chemische Organization der Zelle. Braunschweig.

Pfeffer, Wilhelm

'77 Osmotische Untersuchungen. Leipzig: Engelmann.

'91 Zur Kenntniss der Plasmahaut und der Vacuolen. Abhandlungen der Königlich Sächsischer Gesellschaft der Wissenschaften: Leipzig, pp. 185–344.

Strasburger, Eduard

'91 Das Protoplasma und die Reizharbeit. Jena: Fischer.

Townsend, Ch. O.

'97 Die Einfluss des Zellkerns auf die Bildung der Zellhaut. Jahrb. f. wiss. Botanik, Vol. 30, pp. 484–510.